

1/26

	SampleName	Inj. Volume	Channel	Dilution
1	K63 In PBS	100,00	214nm	4,00
2	K63 In Chaps 0,25%	100,00	214nm	4,00
3	K63 In citrate	100,00	214nm	4,00

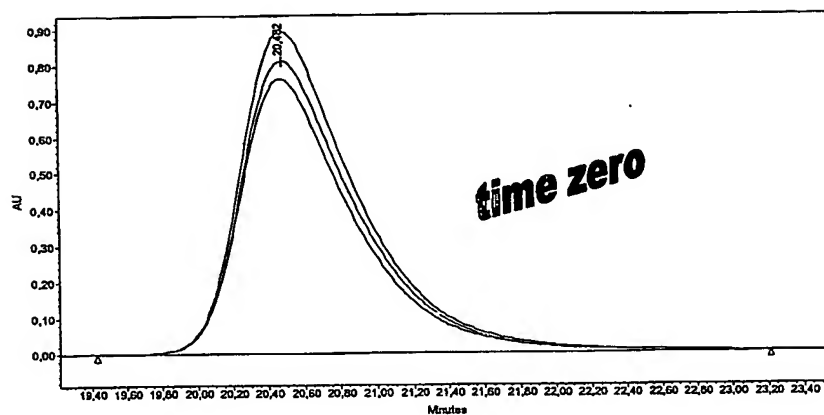


Figure 1A

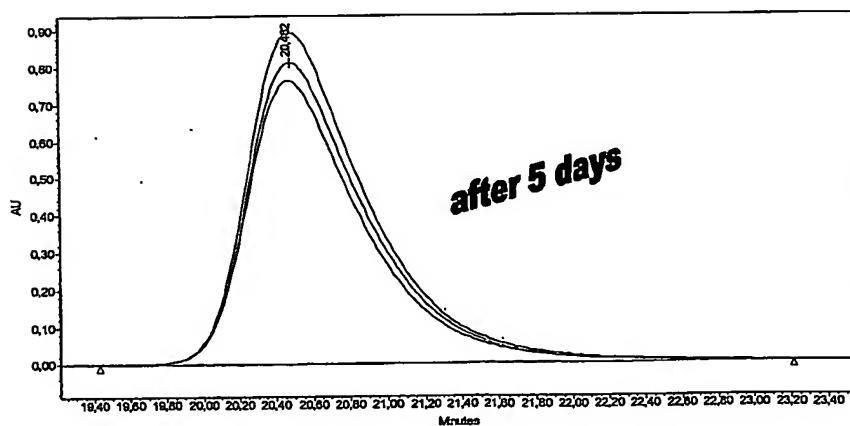
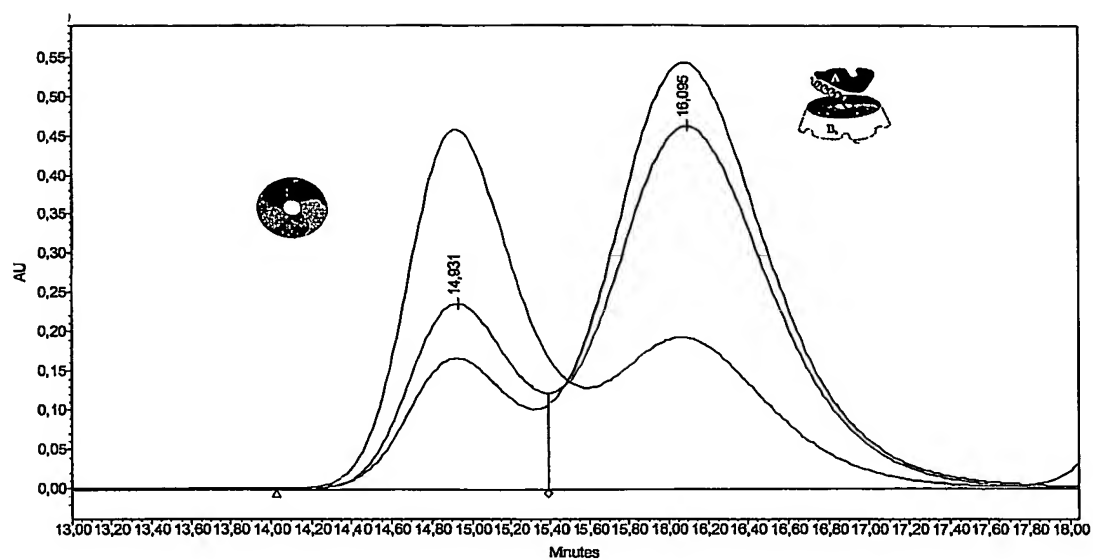


Figure 1B

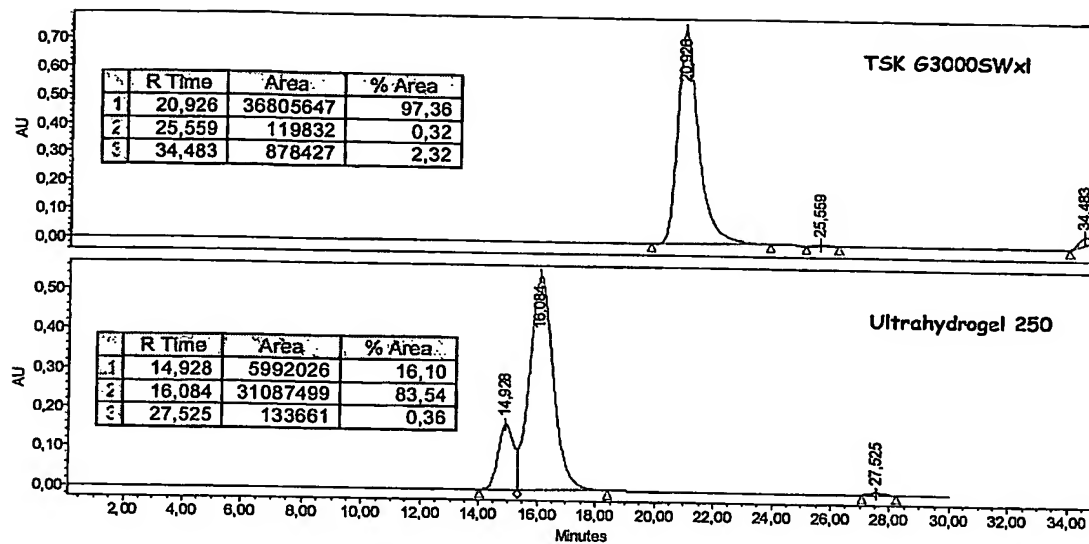
2/26

Figure 1C



3/26

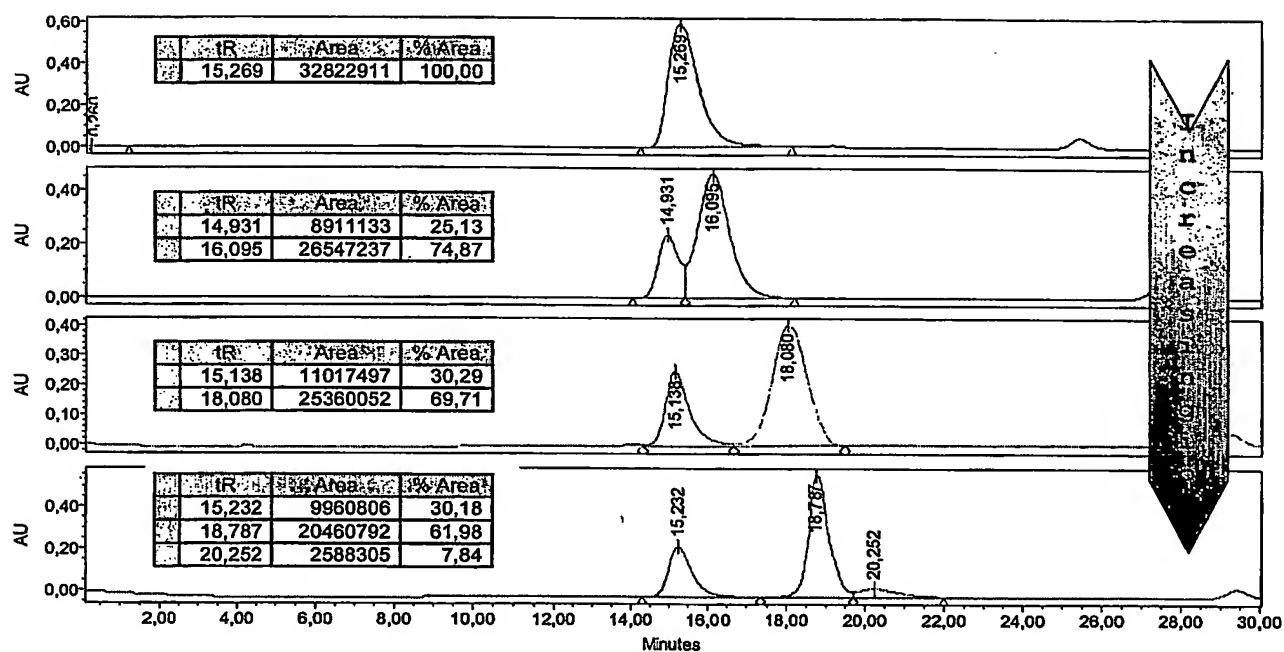
Figure 1D



4/26

Figures 2A-2D

	Sample Name	Date Acquired	Eluent	Injection Volume	Channel	Dilution
1	PBS 5gg agitazione	09/04/2003 9.55.19	KPI 50 mM + Na2SO4 50 mM pH 7,2	100,00	214nm	4,00
2	PBS 5gg agitazione	08/04/2003 13.53.08	KPI 100 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
3	PBS 5gg agitazione	09/04/2003 15.07.11	KPI 250 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
4	PBS 5gg agitazione	10/04/2003 9.51.42	KPI 200 mM + Na2SO4 200 mM pH 7,2	100,00	214nm	4,00



5/26

Figure 3

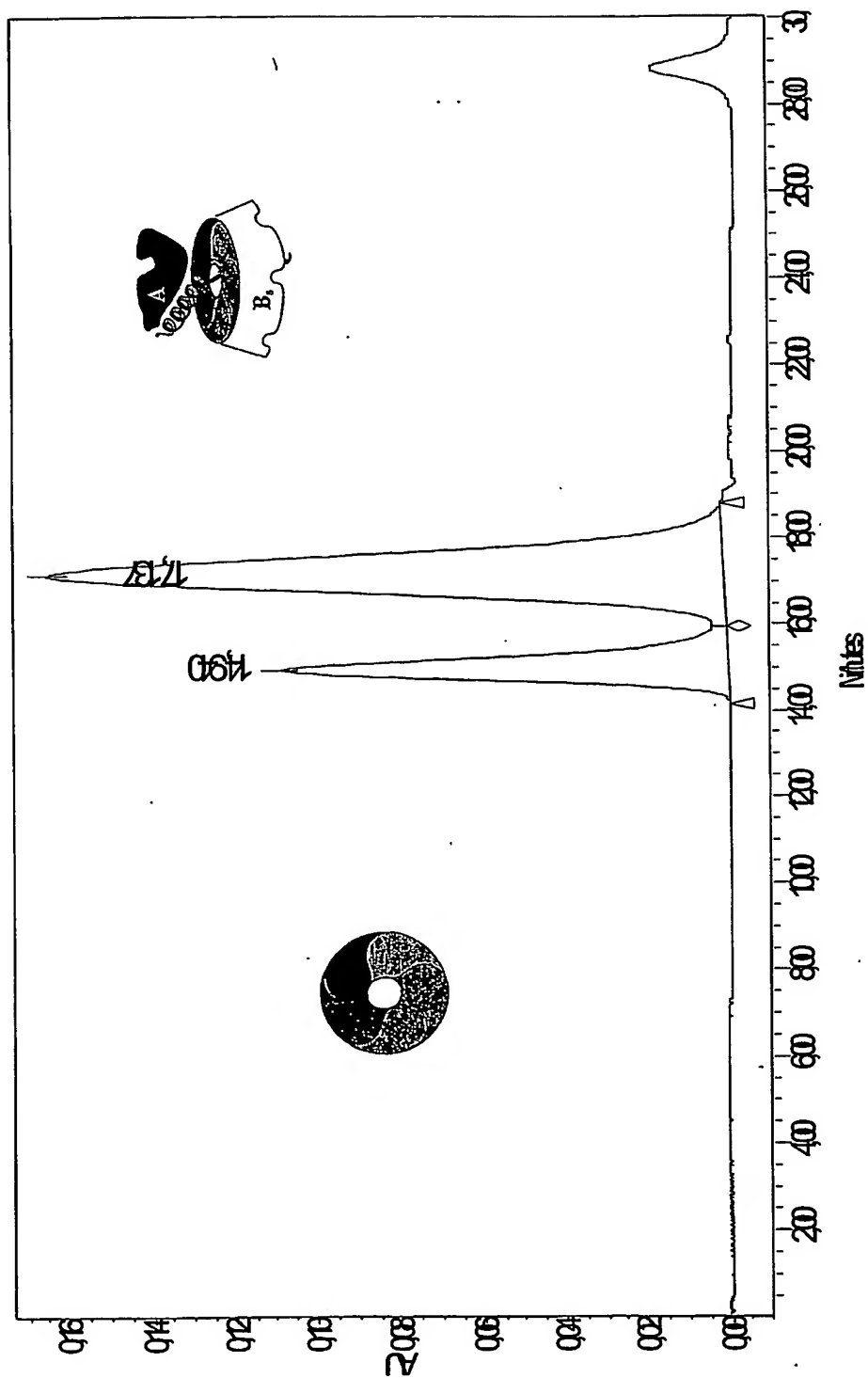
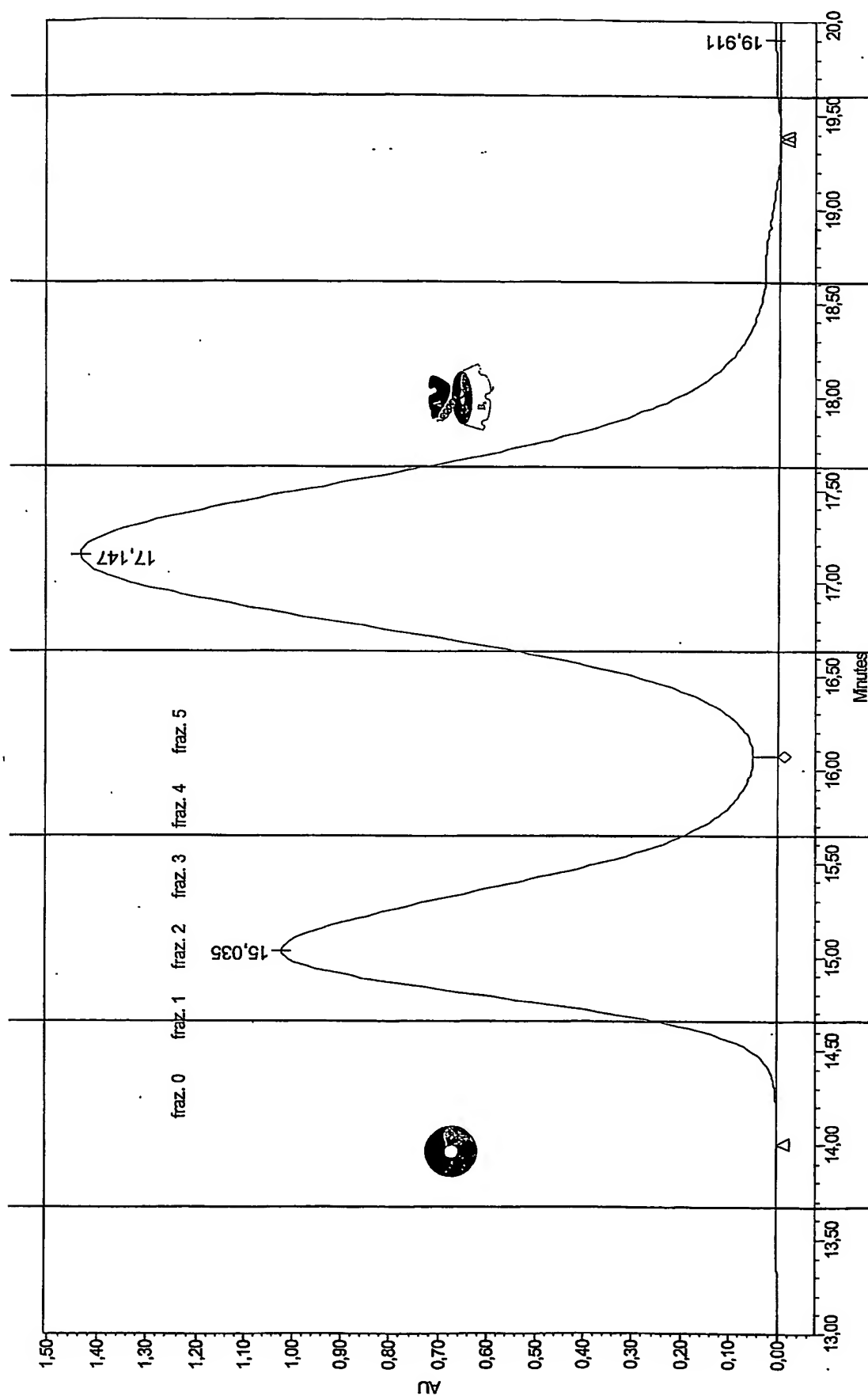


Figure 4



7/26

Figure 5A

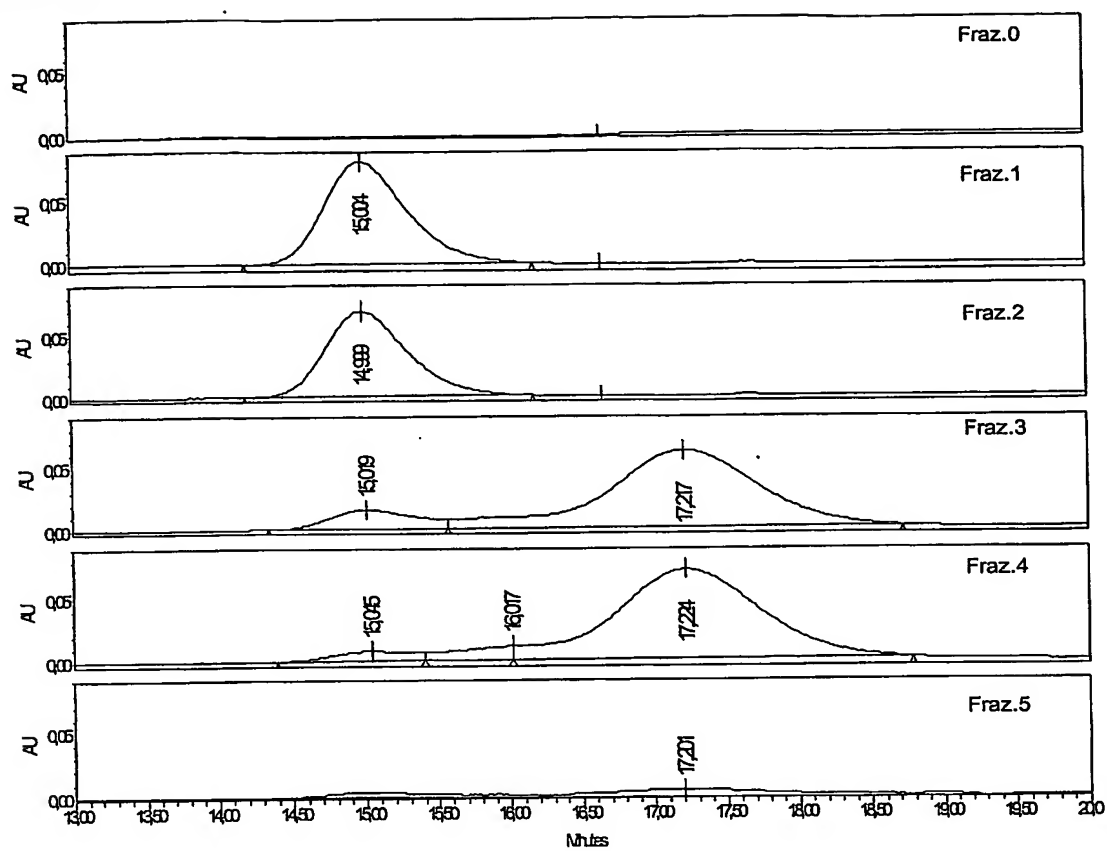


Figure 5B

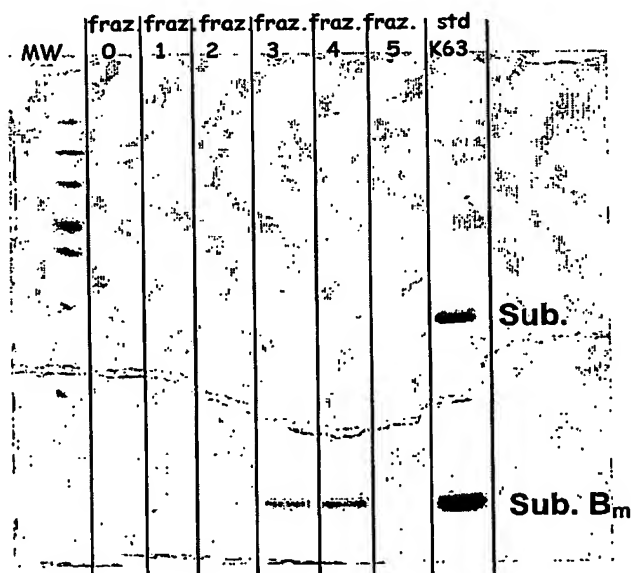


Figure 5C

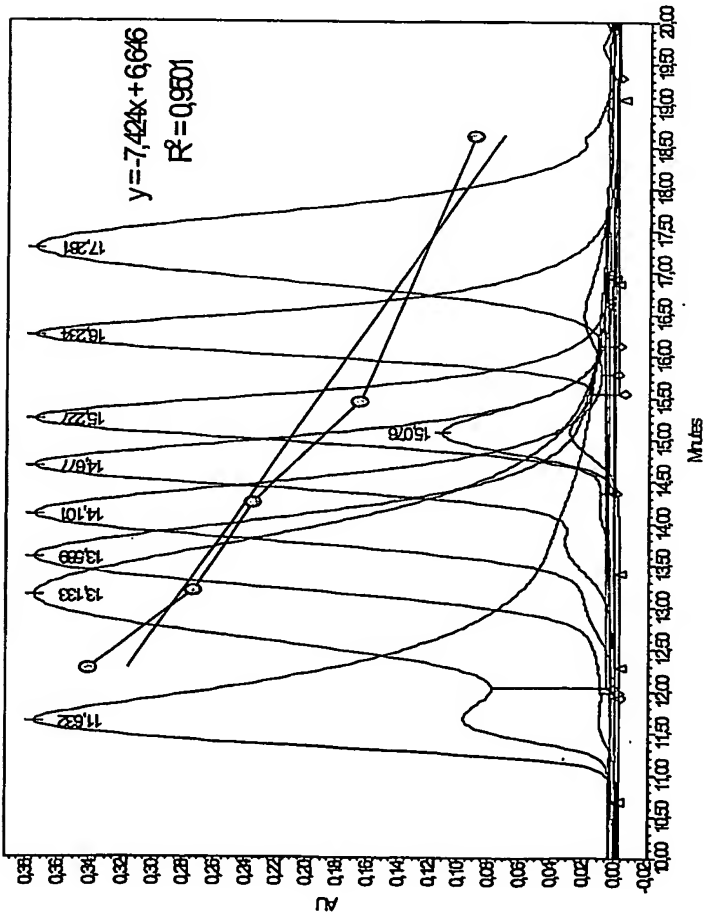


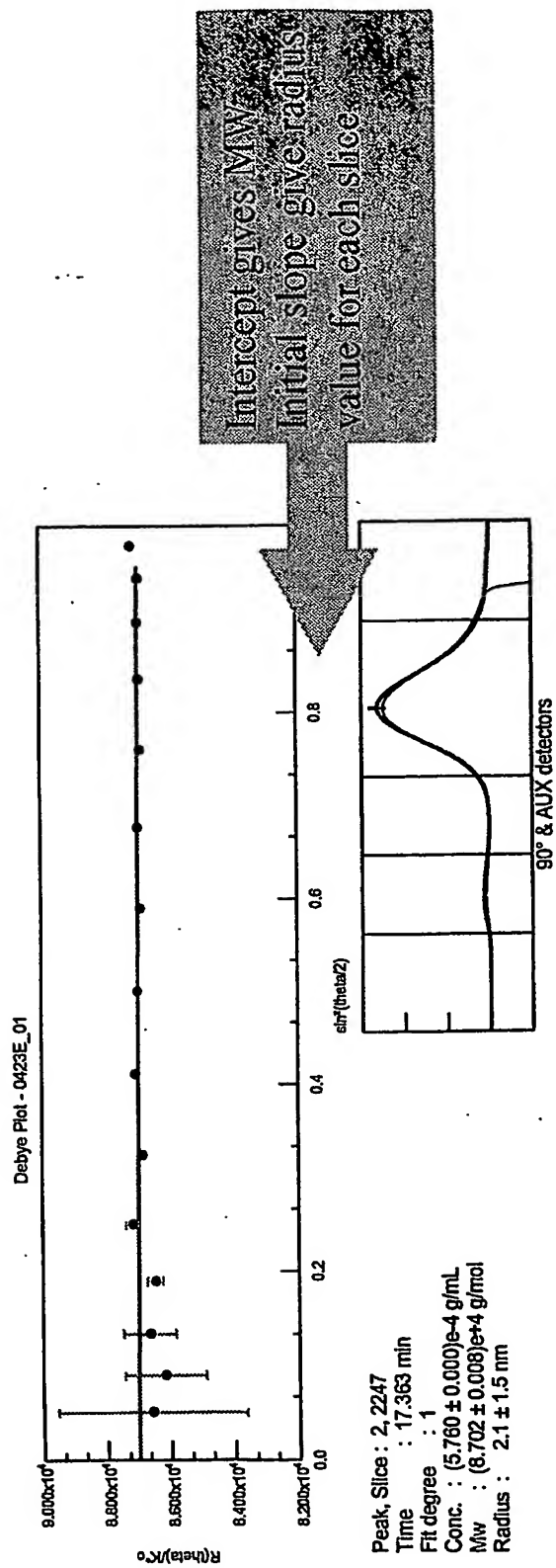
Figure 5D

Standard proteins	Rt (min)	M _w (Da)
Thyroglobulin (bovine)	11.62	669.000
Apoferitin	13.13	476.316
B-amylase	13.58	224.340
Alcohol Deydrogenase	14.10	146.980
BSA	14.67	66.800
Carbonic Anhydrase	16.22	29.023
Sample proteins	Rt (min)	M _w exp.
CRM	15.23	57.099
K63 AB ₅	17.26	9.611
K63 B ₅	15.07	65.607

Superimposition of standard proteins, CRM₁₉₇, reference (bold blue), K63 (bold red) and calibration curve used for apparent MW determination.

9/26

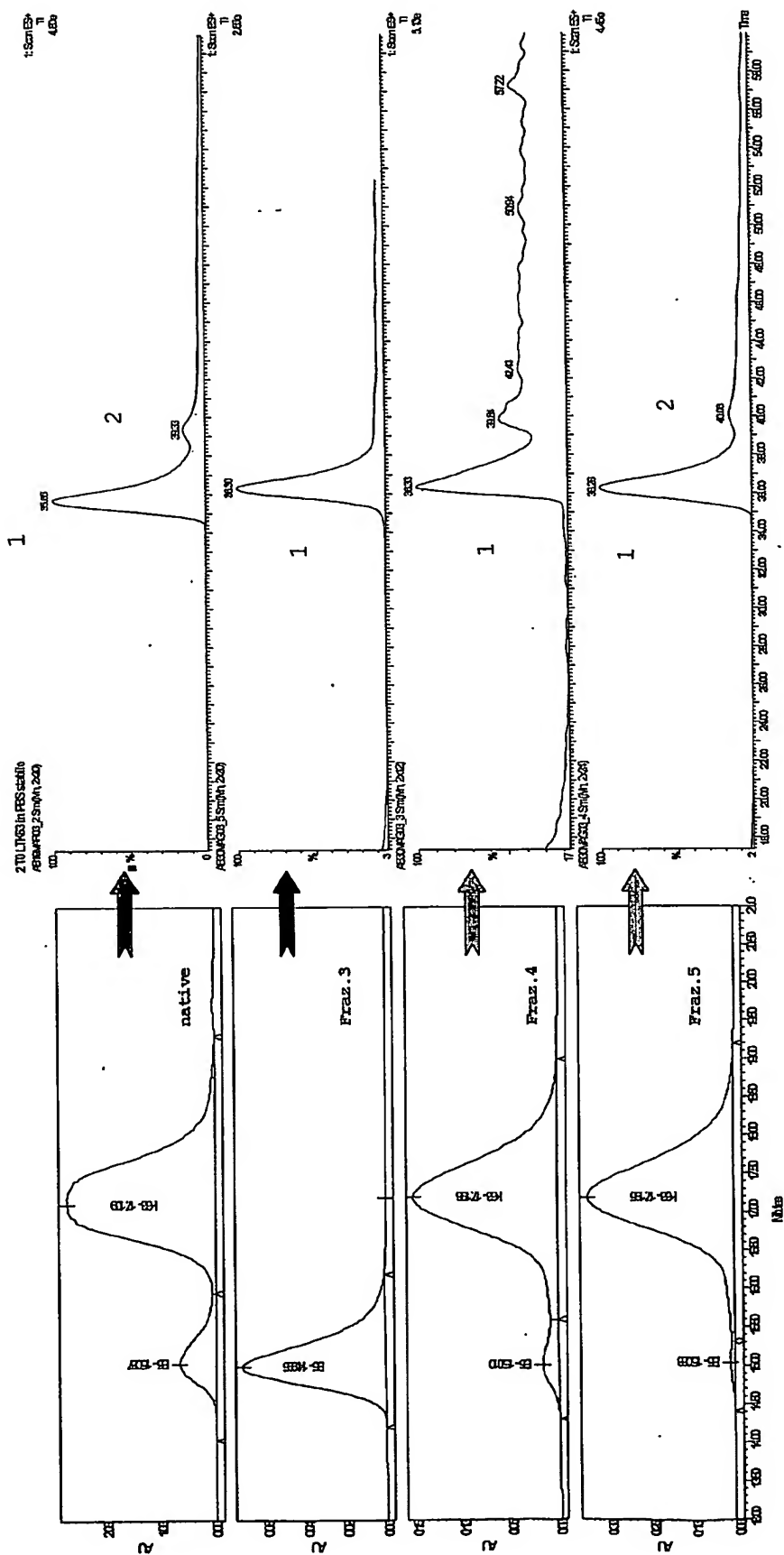
Figure 5E



10/26

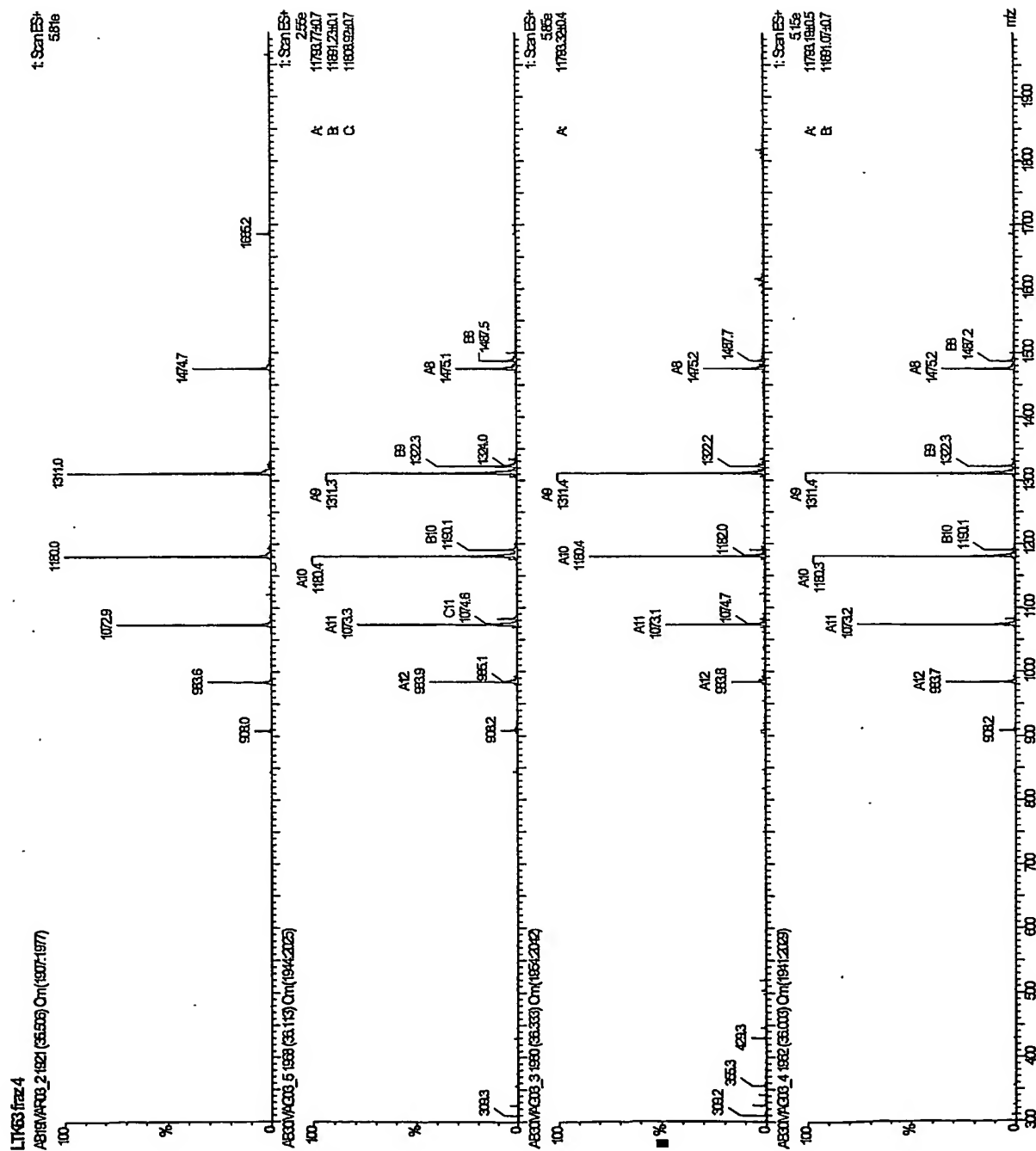
Figure 5F (a)

Figure 5F(b)



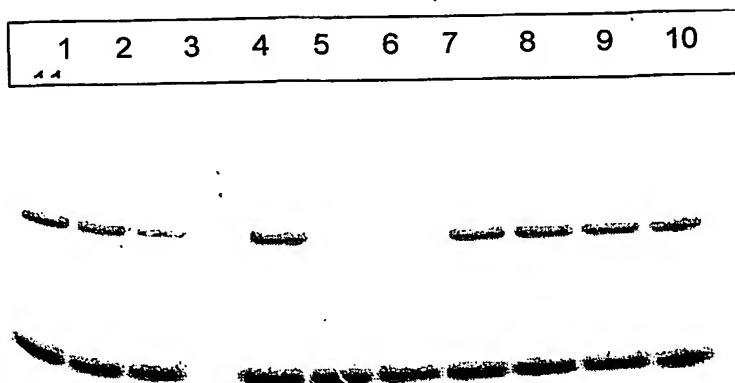
11/26

Figure 5G



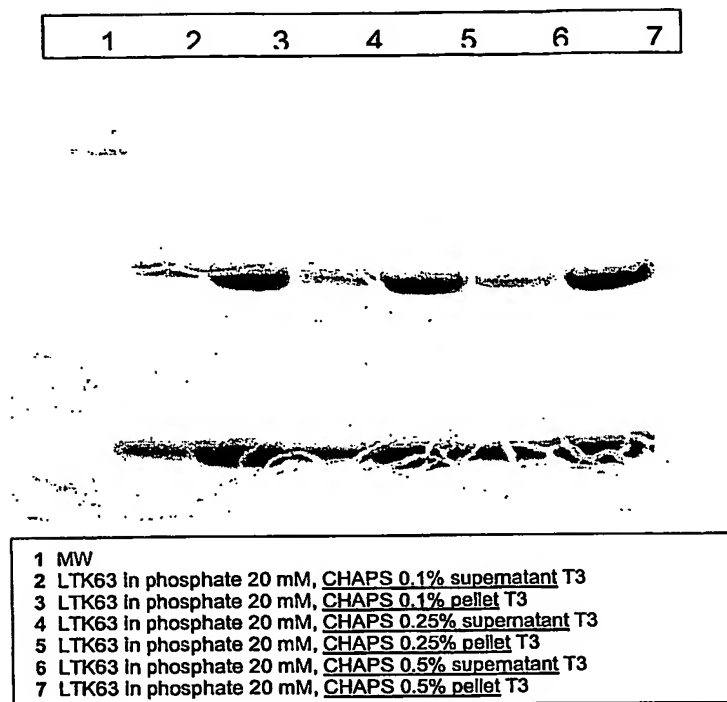
12/26

Figure 6: SDS-PAGE analysis of LTK 63 shaken samples



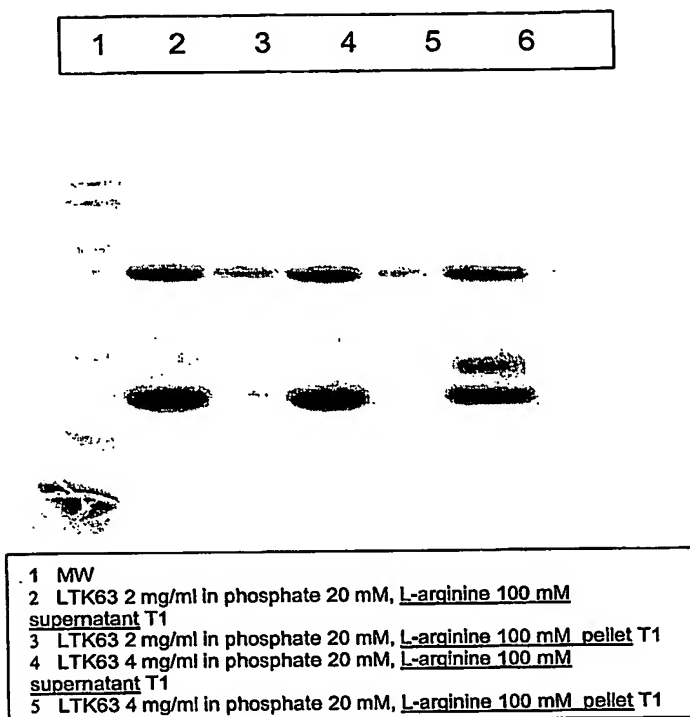
BEST AVAILABLE COPY

13/26

Figure 7: SDS-PAGE analysis of LTK 63 samples treated with CHAPS

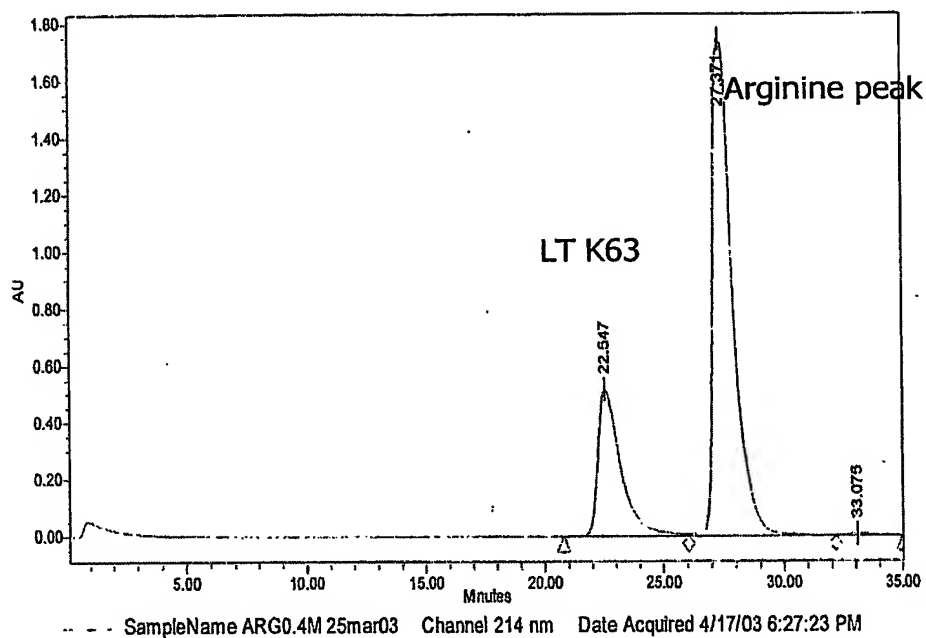
BEST AVAILABLE COPY

14/26

Figure 8: SDS-PAGE of LTK63 samples treated with L-Arginine

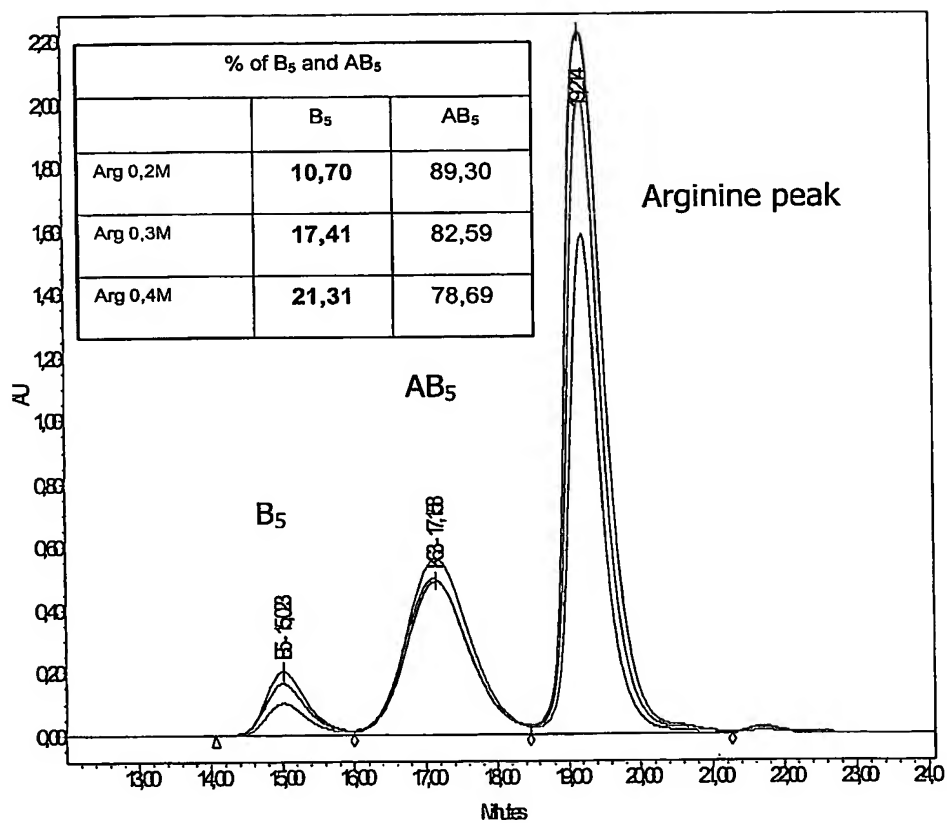
BEST AVAILABLE COPY

15/26

Figure 9(a): Old HPLC Method for analysing L-Arginine treated samples

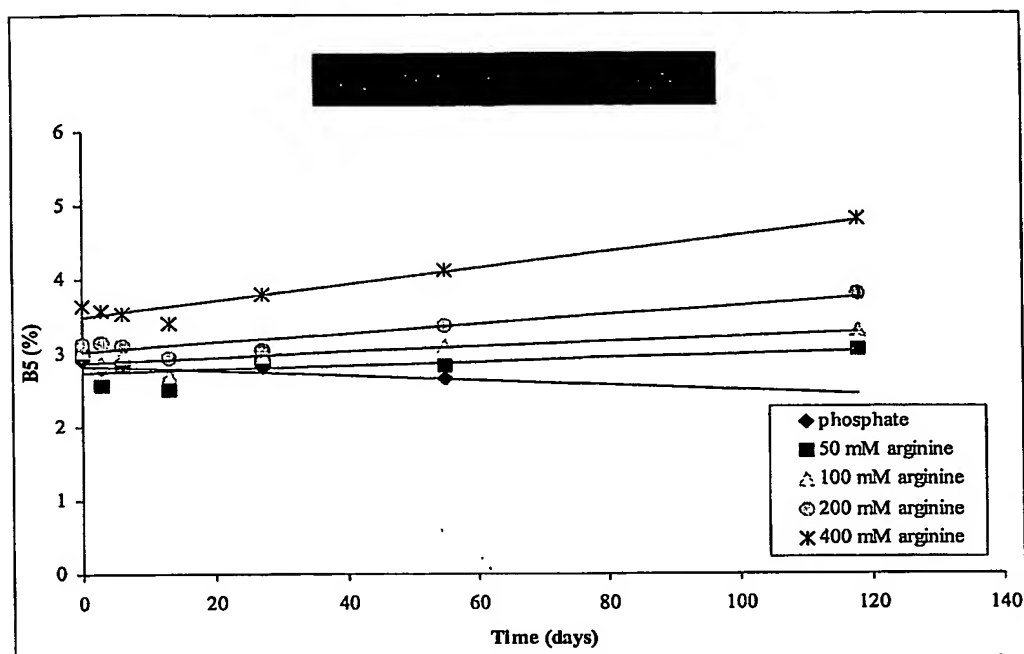
16/26

Figure 9(b): New HPLC Method for analysing L-Arginine treated samples



17/26

Figure 10(a): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 1.3mg/ml



BEST AVAILABLE COPY

18/26

Figure 10(b): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 4.0mg/ml

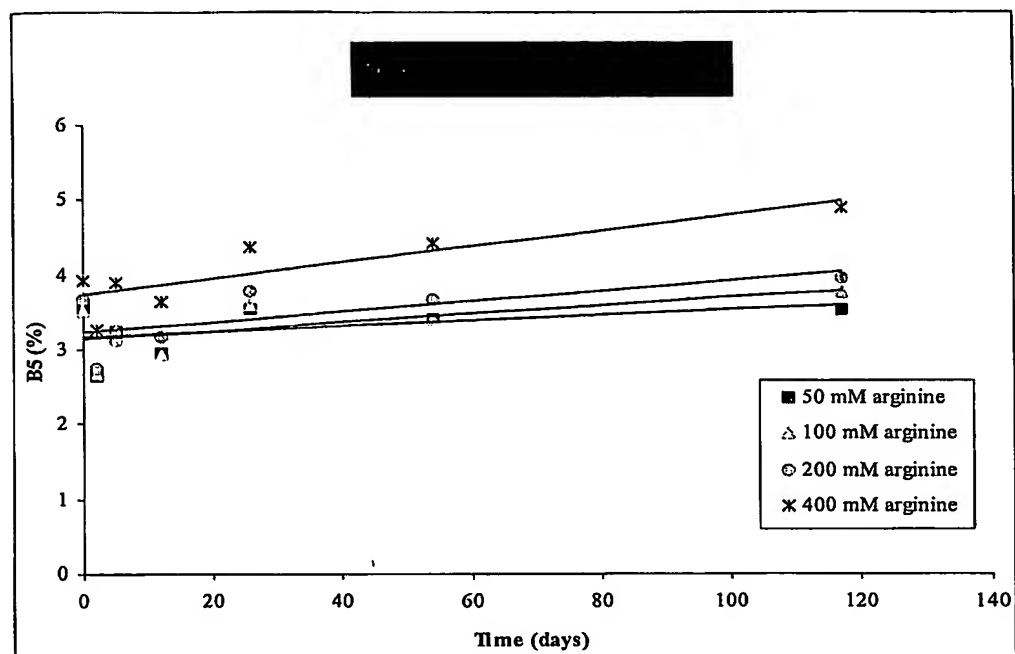
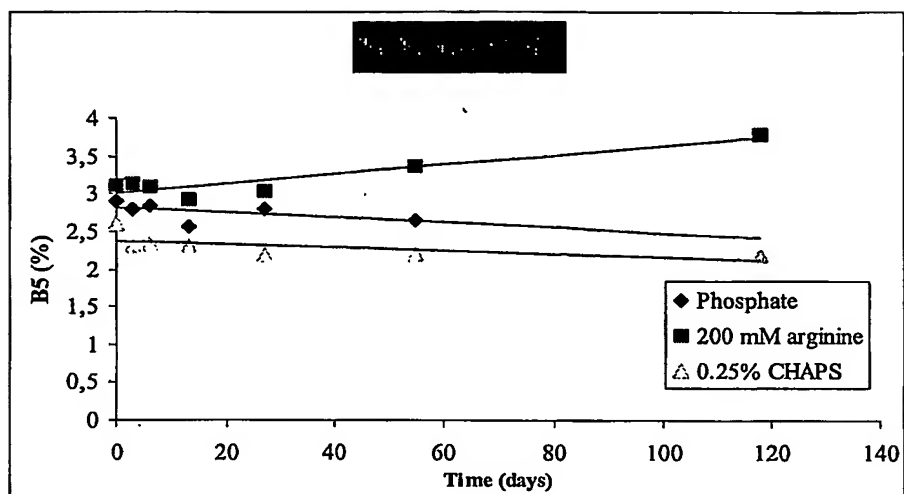


Figure 11(a): CHAPS effect on LTK63 dissociation

20/26

Figure 11(b): CHAPS effect on LT K63 dissociation in combination with L-Arginine

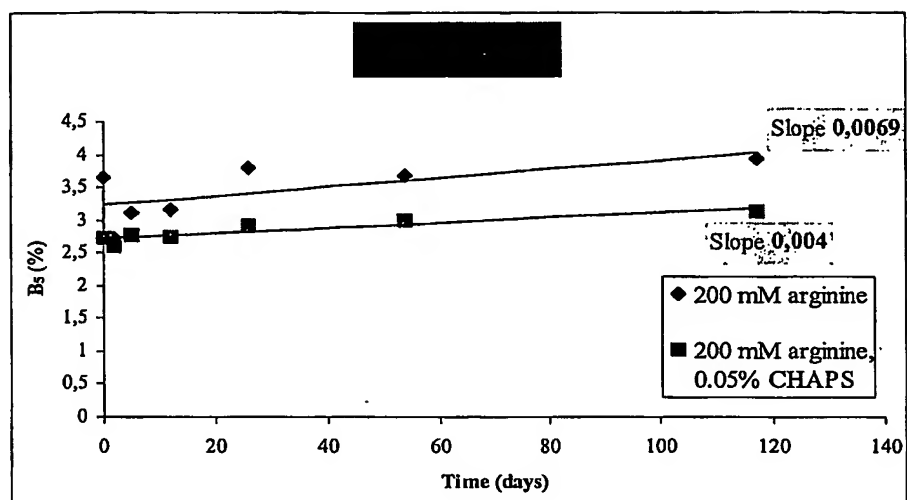


Figure 12: Effect of L-Arginine and CHAPS on LTK 63 stability at a protein concentration of 1,3 mg/ml

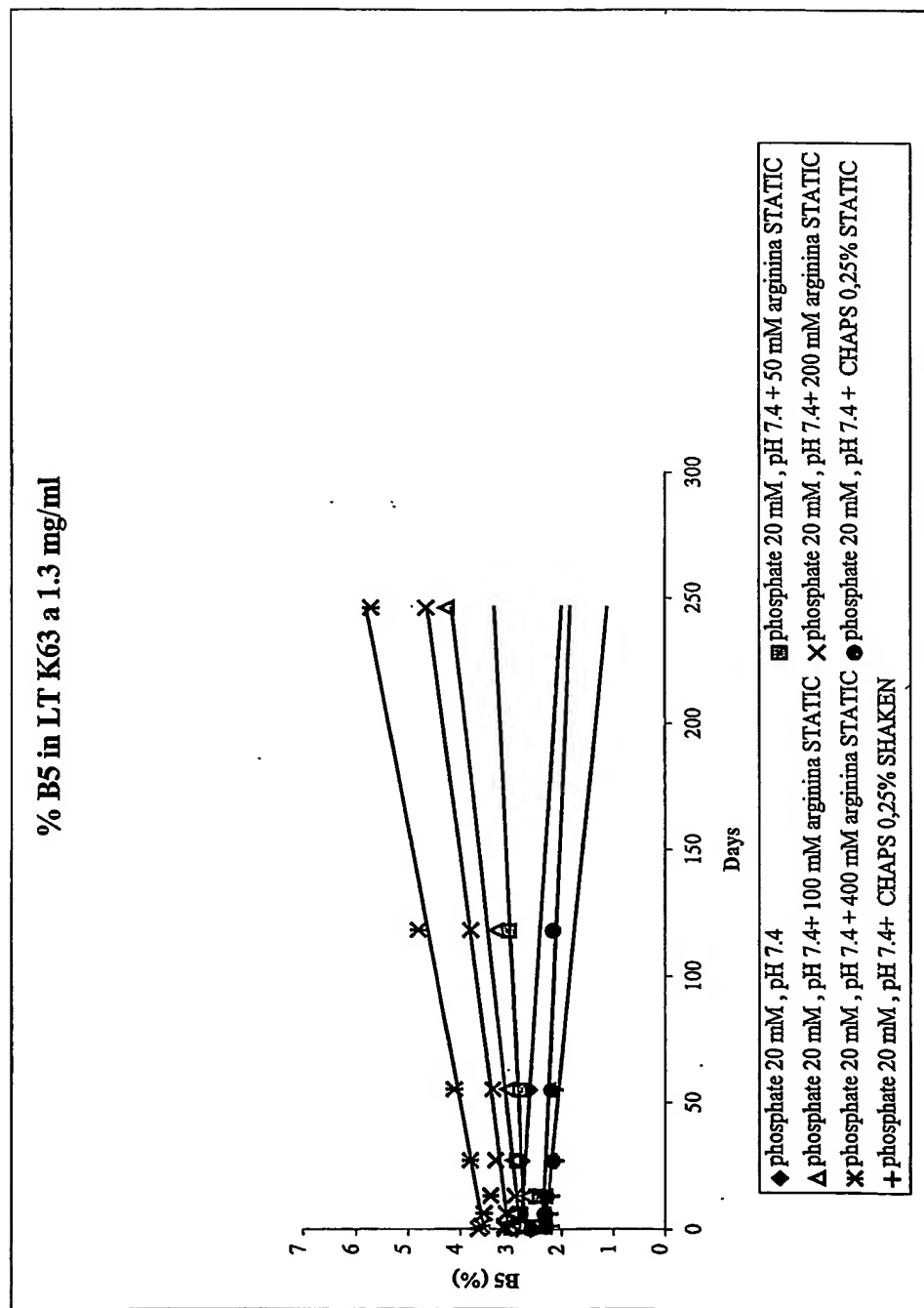
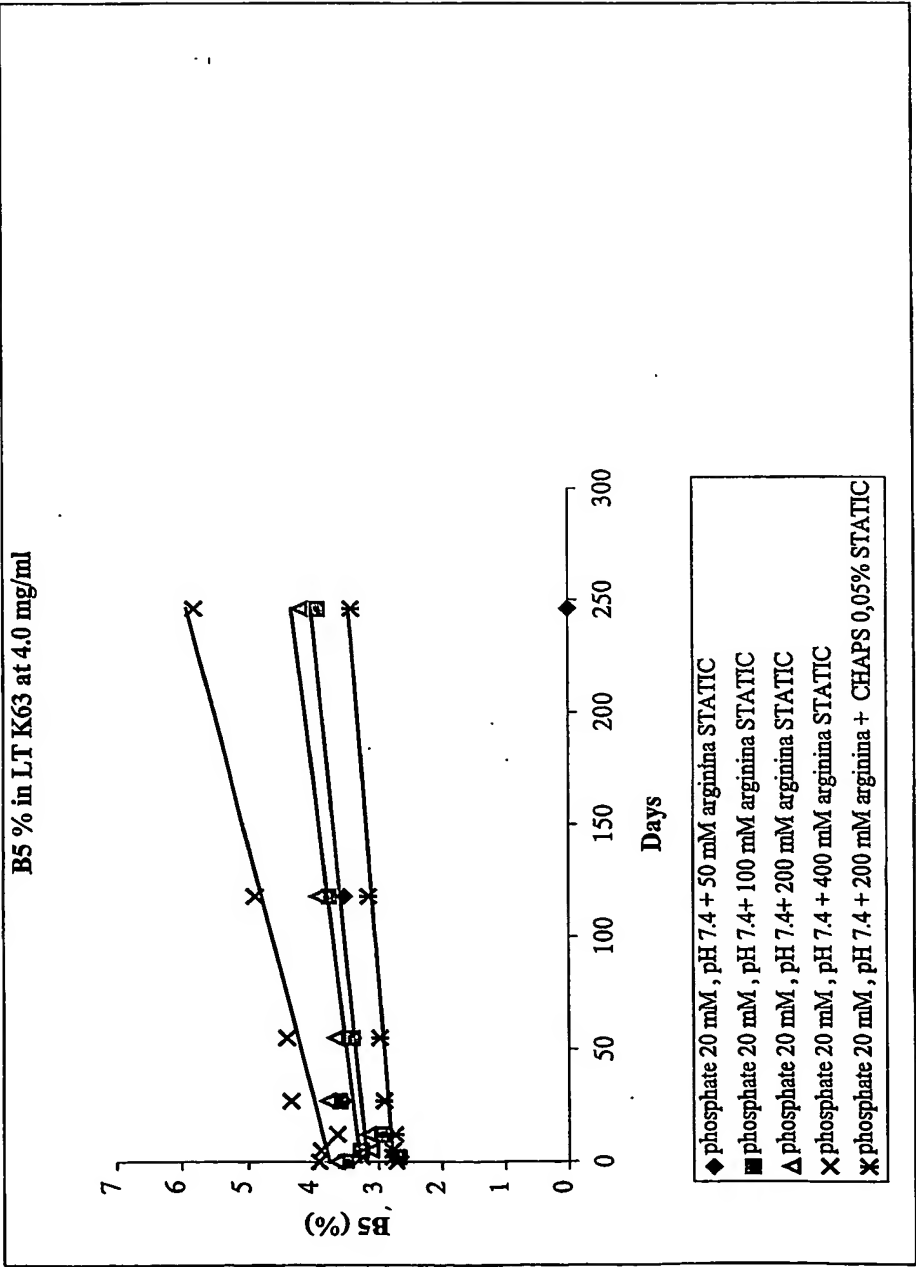


Figure 13: The effect of L-Arginine and the combination L-Arginine/CHAPS on LTK 63 stability at a protein concentration of 4,0 mg/ml



23/26

Figure 14 shows the effect of storage conditions on LTK 63 stability in L-Arginine + CHAPS containing buffers

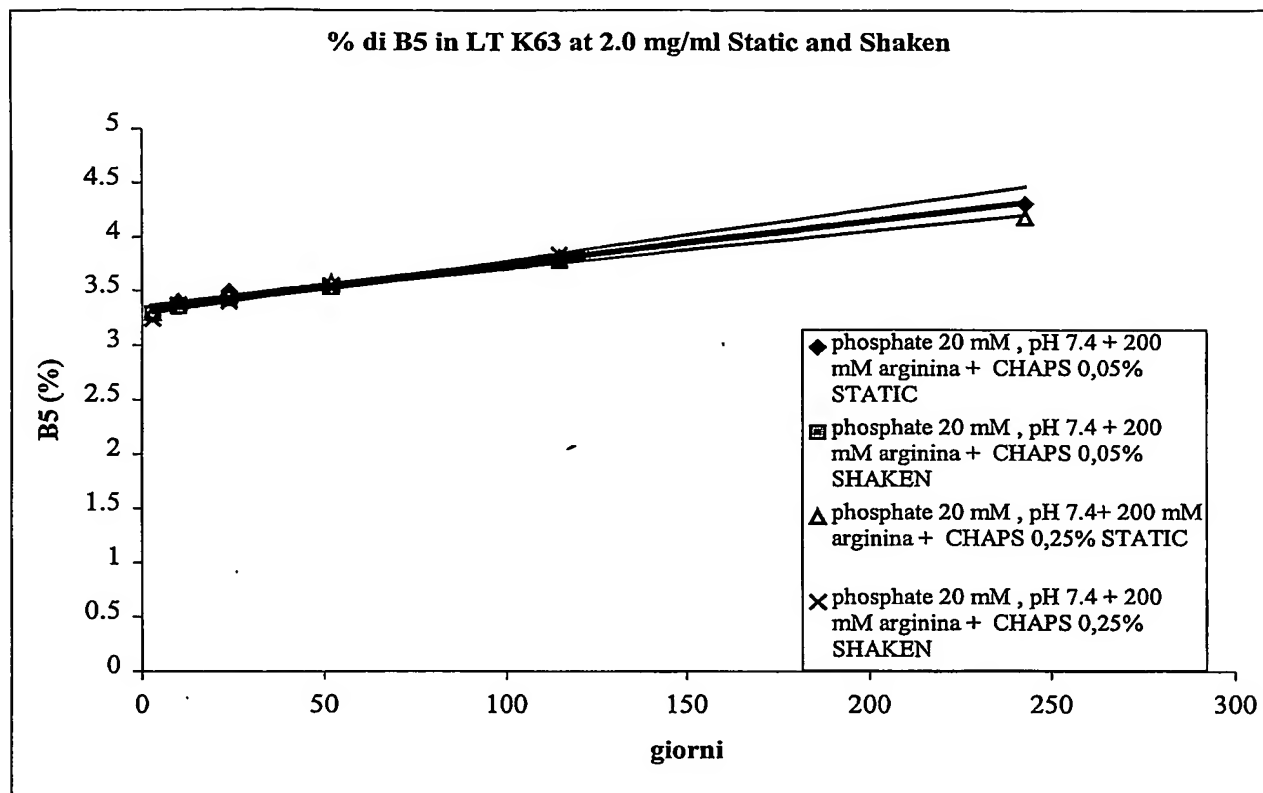


Figure 15: Comparison of LTK 63 stability on L-Arginine and L-Arginine + CHAPS storage buffers

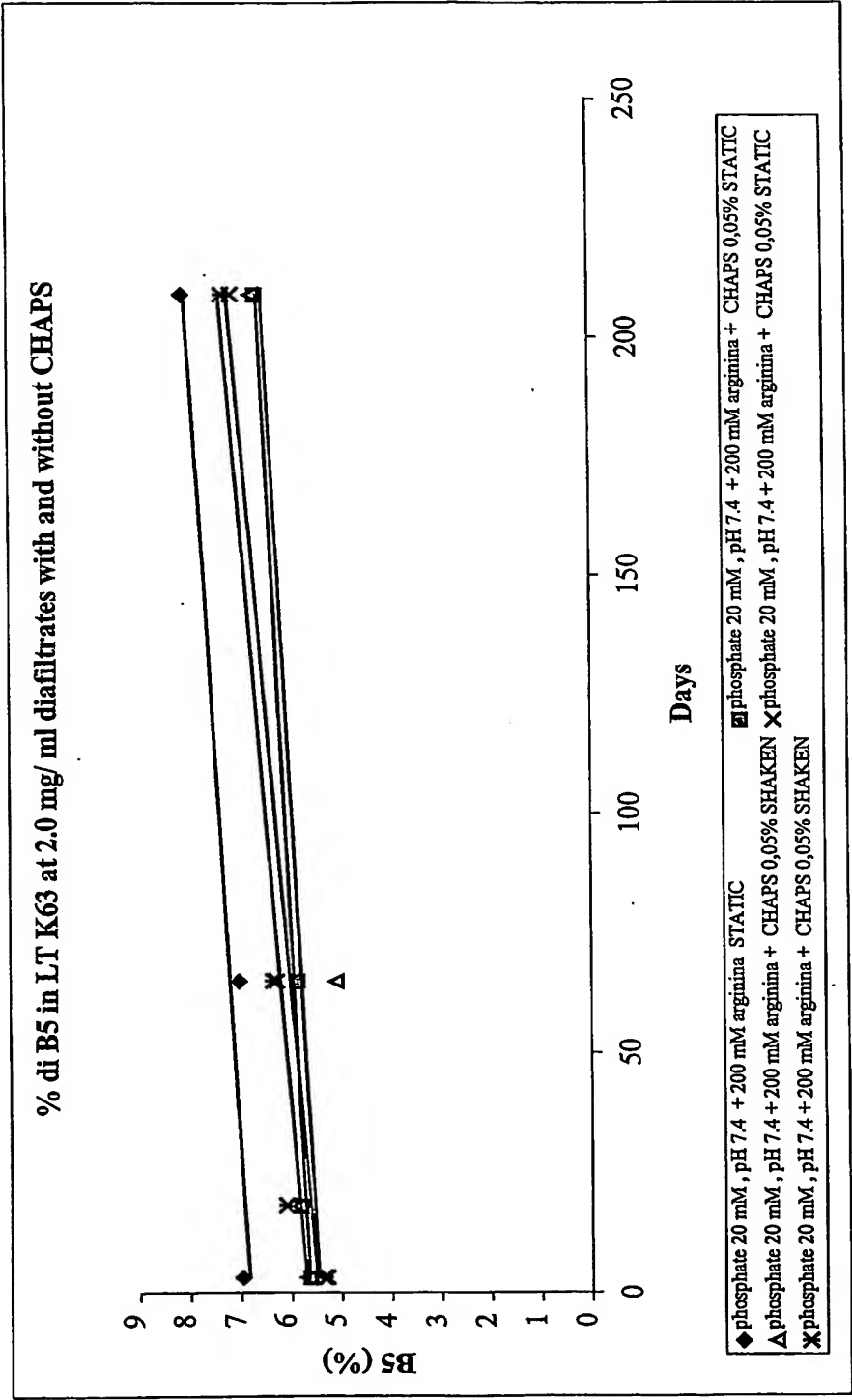


Figure 16

Table 2. Structure and Classification of Detergents (continued)	
<p data-bbox="373 283 406 682">EMPIGEN BB® (n-dodecyl)-N,N-dimethylglycine</p> $\text{CH}_3(\text{CH}_2)_{11}-\text{N}^+(\text{CH}_3)_2-\text{CH}_2-\text{COO}^- \quad \text{pH} \geq 6$ <p data-bbox="438 1218 568 1449"> $\text{CH}_3(\text{CH}_2)_x-\text{N}^+(\text{CH}_3)_2-\text{CH}_2-\text{COO}^-$ </p> <p data-bbox="714 1071 828 1449"> $\text{CH}_3(\text{CH}_2)_x-\text{N}^+(\text{CH}_3)_2-\text{CH}_2-\text{SO}_3^-$ </p> <p data-bbox="1023 777 1299 1533"> </p>	<p data-bbox="698 336 893 682"> x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16 </p> <p data-bbox="1055 483 1120 682"> x = H, CHAPS x = OH, CHAPSO </p>

Zwittergents

26/26

Figure 17

Zwitterionic Detergents

Product	Cat. No.	M.W. (anhydrous)	CMC ^b (mM)	Aggregation No.	Average Micellar Weight	size
ASB-14	182750	434.7	—	—	—	5g, 25 g
ASB-16	182755	462.7	—	—	—	5g, 25 g
CHAPS	220201	614.9	6-10	4-14	6000	1 g 5 g 10 g 25 g
CHAPSO	220202	630.9	8	11	7000	1 g 5 g
DDMAB	252000	299.5	4.3	—	—	5 g
DDMAUS	252005	319.7	0.1	—	—	5 g
EMPIGEN BB [®] Detergent, 30% Solution	324690	272.0	1.6-2.1	—	—	100 ml
Lauryldimethylamine Oxide (LDAO) 30% Solution	498011	279.4	1.2	76	17,000	100 ml
ZWITTERGENT [®] 3-08 Detergent	693019	279.6	330	—	—	5 g
ZWITTERGENT [®] 3-10 Detergent	693021	307.6	25-40	—	12,500	5 g 25 g
ZWITTERGENT [®] 3-12 Detergent	693015	335.6	2-4	55	18,500	5 g 25 g
ZWITTERGENT [®] 3-14 Detergent	693017	363.6	0.1-0.4	183	30,000	5 g 25 g
ZWITTERGENT [®] 3-16 Detergent	693023	391.6	0.01-0.06	155	60,000	5 g 25 g

a. Average molecular weights are given for detergents composed of mixtures of chain lengths; b. Temperature: 20 - 25°C

TRADEMARKS

BRU[®] and TWEEN[®] are registered trademarks of ICI Americas, Inc.
 EMPIGEN BB[®] is a registered trademark of Albright & Wilson.
 GENAPOL[®] is a registered trademark of Hoechst AG.
 ULTRAL[®], PROTEIN-GRADE[®] and ZWITTERGENT[®] are registered trademarks of Calbiochem-Novabiochem Corporation.
 CALBIOSORB[™], Adsorbent and ELUGENT[™] Detergent are trademarks of Calbiochem-Novabiochem Corporation

LUBROL[®] is a registered trademark of Imperial Chemical Inc.
 PLURONIC[®] is a registered trademark of Wyandotte Chemicals Corporation.
 TRITON X[®] is a registered trademark of Rohm and Haas.

BEST AVAILABLE COPY